

[First Hit](#) [Fwd Refs](#)[Previous Doc](#)[Next Doc](#)[Go to Doc#](#)

End of Result Set



Generate Collection

Print

L2: Entry 1 of 1

File: USPT

Jan 6, 2004

US-PAT-NO: 6673556

DOCUMENT-IDENTIFIER: US 6673556 B2

TITLE: Methods of detecting specific cell lysis

DATE-ISSUED: January 6, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Nixon; Douglas	San Francisco	CA		
McDermott; Adrian B.	Leyburn			GB
Furlan; Scott	San Francisco	CA		
Bigos; Martin	San Francisco	CA		
Sheehy; Megan	Syracuse	NY		
Klenerman; Paul	Oxford			GB

US-CL-CURRENT: 435/7.2; 435/4, 435/40.5, 435/7.21

CLAIMS:

What is claimed is:

1. A method of detecting specific lysis of a target cell, comprising: a) contacting a labeled viable target cell with a lytic agent, wherein the target cell is labeled with a first plasma membrane-labeling fluorescent dye that labels the plasma membrane of the target cell and a first cytosol-labeling fluorescent dye that labels the cytosol of the target cell; and b) determining the amount of the cytosol-labeling fluorescent dye remaining in the target cell, wherein a reduction in the amount of the cytosol-labeling fluorescent dye in the target cell when the target cell is contacted with the lytic agent, compared to the amount of cytosol-labeling fluorescent dye in a control target cell not contacted with the lytic agent, indicates that the target cell is lysed by the lytic agent, and wherein said first plasma membrane-labeling fluorescent dye has detectably different spectral properties from the first cytosol-labeling fluorescent dye such that the first plasma membrane-labeling fluorescent dye is distinguishable from the first cytosol-labeling fluorescent dye.

2. The method of claim 1, wherein said first plasma membrane-labeling fluorescent dye is a lipid-associated fluorescent dye, and wherein said first cytosol-labeling fluorescent dye is a fluorescent dye that labels proteins in the cytosol.

3. The method of claim 1, wherein the lytic agent is a cell having lytic activity toward the target cell.

4. The method of claim 3, wherein the cell having lytic activity toward the target cell is an antigen-specific CD8.sup.+ T lymphocyte, and the target cell displays the antigen in an MHC Class I molecule on its cell surface.
5. The method of claim 1, wherein the lytic agent is an antibody specific for a cell surface marker on the target cell.
6. The method of claim 1, wherein the lytic agent comprises an antibody specific for a cell surface marker on the target cell, and a lytic cell having an Fc receptor, wherein the lytic cell is selected from the group consisting of a neutrophil, an eosinophil, a macrophage, a monocyte, and a natural killer cell.
7. The method of claim 1, wherein the emission of the first plasma membrane-labeling fluorescent dye differs from the emission of the first cytosol-labeling fluorescent dye by at least about 10 nm.
8. The method of claim 2, wherein the lipid-associated fluorescent dye is selected from PKH-26, PKH-67, and a long chain dialkylcarbocyanine.
9. The method of claim 2, wherein the protein-labeling cytosol dye is selected from 5-(-6)-carboxyfluorescein, 5-(-6) (((4-chloromethyl)benzoyl)amino) tetramethylrhodamine), 7-amino-4-chloromethylcoumarin, and a SNARF.RTM. fluorescent dye.
10. The method of claim 1, wherein the amount of cytosol-labeling fluorescent dye in the target cell is determined using flow cytometry.

[Previous Doc](#)[Next Doc](#)[Go to Doc#](#)